Claims, PCT/EP02/14895.

1. A method for obtaining a pluripotent human blastocyst-derived stem cell line, the method comprising the steps of

i) using a fertilized oocyte, having a grade 1 or 2, to obtain a blastocyst, having a grade A or B,

ii) co-culturing the blastocyst with feeder cells for establishing one or more colonies of inner cell mass cells,

iii) isolating the inner cell mass cells by mechanical dissection,

iv) co-culturing of the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line.

v) optionally, propagation of the blastocyst-derived stem cell line.

2. A method for obtaining a pluripotent human blastocyst-derived stem cell line, the method comprising the steps of

i) using a fertilized oocyte having a grade 1 or 2, to obtain a blastocyst, optionally having a grade A or B,

ii) co-culturing the blastocyst with feeder cells for establishing one or more colonies of inner cell mass cells,

iii) isolating the inner cell mass cells by mechanical dissection,

iv) co-culturing of the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line.

v) optionally, propagation of the blastocyst-derived stem cell line.

3. A method for obtaining a pluripotent human blastocyst-derived stem cell line, the method comprising the steps of

i) using a fertilized oocyte optionally, having a grade 1 or 2, to obtain a blastocyst, having a grade A or B,

ii) co-culturing the blastocyst with feeder cells for establishing one or more colonies of inner cell mass cells,

iii) isolating the inner cell mass cells by mechanical dissection,

iv) co-culturing of the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line.

v) optionally, propagation of the blastocyst-derived stem cell line.

4. A method for obtaining a pluripotent human blastocyst-derived stem cell line, the method comprising the steps of

i) using a fertilized oocyte optionally, having a grade 1 or 2, to obtain a blastocyst, optionally having a grade A or B,

ii) co-culturing the blastocyst with feeder cells for establishing one or more colonies of inner cell mass cells,

iii) isolating the inner cell mass cells by mechanical dissection,

iv) co-culturing of the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line,

v) propagation of the blastocyst-derived stem cell line culturing the stem cells with feeder cells of a density of less than about 60,000 cells per cm^2, such as e.g. less than about 55,000 cells per cm^2, or less than about 50,000 cells per cm^2, such as about 45,000 cells per cm^2.

5. A method according to any of the claims 1-4 in which the blastocyst in step i) is a spontaneously hatched blastocyst.

6. A method according to any of the claims 1-5 in which the blastocyst-derived stem cell line is stable.

7. A method according to any of the claims 1-6 wherein the blastocyst-derived stem cell line is propagated.

8. A method according to claim 7, in which the propagation of blastocyst-derived stem cell line comprises passage of the stem cell line every 4-5 days.

9. A method according to claims 7-8, in which the propagation of blastocyst-derived stem cell line comprises culturing the stem cells with feeder cells of a density of less than about 60,000 cells per cm^2, such as e.g. less than about 55,000 cells per cm^2, or less than about 50,000 cells per cm^2.

10. A method according to claim 9, in which the propagation of blastocyst-derived stem cell line comprises culturing the stem cells with feeder cells of a density of about 45,000 cells per cm^2.

11. A method according to claims 7-10, in which the propagation of blastocyst-derived stem cell line comprises passage of the feeder cells at the most 3 times, such as e.g. at the most 2 times.

12. A method according to any of the claims 1-11 in which the zona pellucida of the blastocyst has been at least partially digested prior to step ii).

13. A method according to claim 12 in which the zona pellucida of the blastocyst has been at least partially digested with a digestive agent selected from the group comprising acidic reacting substances, enzymes and mixtures thereof.

14. A method according to any of the claims 1-13 in which step ii) and/or step iv) is performed in an agent that improves the attachment of the blastocysts and/or if relevant the inner cell mass cells to the feeder cells.

15. A method according to claim 14 wherein the agent is a hyaluronic acid.

16. A method according to any of the claims 1-15 in which the feeder cells are embryonic feeder cells.

17. A method according to any of the claims 1-16 in which the feeder cells employed in steps ii) and iv) are the same or different and originate from animal source.

18. A method according to claim 17 wherein the feeder cells are of mouse or human origin.

19. A method according to any of the claims 1-18, wherein the feeder cells are mitotically inactivated.

20. A method according to any of the claims 1-19, wherein the stem cell line

i) exhibits proliferation capacity in an undifferentiated state for more than 21 months when grown on mitotically inactivated embryonic feeder cells, and

ii) exhibits normal euploid chromosomal karyotype, and

iii) maintains potential to develop into derivatives of all types of germ layers both *in vitro* and *in vivo*, and

iv) exhibits at least two of the following molecular markers OCT-4, alkaline phosphatase, the carbohydrate epitopes SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, and the protein core of a keratin sulfate/chondroitin sulfate pericellular matrix proteinglycan recognized by the monoclonal antibody GCTM-2, and

v) does not exhibit molecular marker SSEA-1 or other differentiation markers, and

vi) retains its pluripotency and forms teratomas in vivo when injected into immuno-compromised mice, and

vii) is capable of differentiate.

21. Use of the human blastocyst-derived stem cell line obtained by the method according to any of the claims 1-20 for the preparation of differentiated cells.

22. A method according to any of the claims 1-20, wherein the stem cell line has the ability of differentiating into an insulin producing cells.

23. A method according to claim 22, wherein the insulin producing cells are capable of forming islet-like structures.

24. A method according to claims 22 or 23, wherein the amount of insulin producing β-cells which are derived from the pluripotent human BS cell line is higher than 25%, such as e.g. higher than 35%, or higher than 40%, or higher than 45%, or higher than 50%.

25. A method according to claims 22-24, wherein the insulin producing cell line produces at least about 300 ng insulin/mg total protein such as at least about 380 ng insulin/mg total protein or at least about 450 ng insulin/mg total protein.

26. A method according to any of the claims 1-20 or 22-25, wherein the blastocyst-derived stem cells have the ability to differentiate into differentiated cells, which display the expression of pancreatic cell type markers, including at least one of insulin, Glut-2, Pdx-1, glucokinase, glucagon and somatostatin.

27. A method according to any of the claims 1-20 or 22-26, wherein the blastocyst-derived stem cells have the ability to differentiate into insulin-producing cells characterized by their organization into islet-like structures comprising an inner core of β-cells surrounded by an outer layer of neuron-type cells, which neuron-type cells

display expression of at least one of the following neuronal cell type markers, including neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase and Map 2.

28. A method according to any of the claims 1-20, wherein the blastocyst-derived stem cells are capable of being made into differentiated cells, which display the expression of at least one of the following neuronal cell type markers, including neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase and Map 2.

29. Use of a preparation of differentiated cells derived from the blastocyst-derived stem cells obtained by the method according to any of the claims 1-20 or 22-28 for the manufacture of a medicament for the prevention or treatment of pathologies or diseases caused by tissue degeneration.

30. Use of a preparation of differentiated cells derived from the blastocyst-derived stem cells obtained by the method according to any of the claims 1-20 or 22-27 for the manufacture of a medicament for the prevention or treatment of pathologies or diseases in the pancreas.

31. Use according to claim 30, in which the disease is diabetes.

32. Use according to claim 28 or 29, in which the disease is type 1 diabetes.

33. Use of a preparation of differentiated cells derived from the blastocyst-derived stem cell line obtained by the method according to any of the claims 1-20 or 28 for the manufacture of a medicament for the prevention or treatment of pathologies or diseases in the nervous system.

34. Use according to claim 33, in which the disease is selected from the group consisting of multiple schlerosis, spinal chord injury, encephalopathies, Parkinson's disease, Huntingdon's disease, stroke, traumatic brain injuries, hypoxia induced brain injuries, ischemia induced brain injuries, hypoglycemic brain injuries, degenerative disorders of the nervous system, brain tumors and neuropathies in the peripheral nervous system.

35. A kit for performing the method according to any of the claims 1-20, comprising human blastocysts with an intact zona pellucida or spontaneously hatched blastocysts and at least two of the following components in separate compartments; hyaluronic acid, pronase, BS-cell medium, and human or mouse embryonic feeder cells.

36. A method for producing an essentially pure preparation of insulin-producing differentiated stem cells, comprising the steps of;

i) expanding human blastocyst-derived stem cells by growing these on an inactivated feeder cell layer in a suitable medium;

ii) generating blastocyst-derived stem cell bodies by dissociating colonies formed in step i) into smaller aggregates or individual cells, followed by transferring said aggregates or individual cells to non-adherent containers where they are incubated in a suitable medium;

iii) plating the blastocyst-derived stem cell bodies in containers in a suitable medium;

iv) selecting nestin-positive neural precursors in ITFSn medium;

v) expanding pancreatic endocrine progenitor cells in, N2-medium comprising B27 media complement and basic fibroblast growth factor;

vi) changing the medium to a basic fibroblast growth factor-free N2 medium.

37. A method according to claim 36 in which the human blastocyst-derived stem cells are obtained by the method according to any of the claims 1-20.

38. A method according to claims 36-37 in which the medium used in step i) is human blastocyst-derived stem cell medium.

39. A method according to claim 36-38 in which the medium used in step ii) is blastocyst-derived stem cell body medium.

40. A method according to claims 36-39 in which the medium used in step iii) is blastocyst-derived stem cell body medium.

41. A method according to claims 36-40 in which nicotinamide is added after step vi).

42. An essentially pure preparation of differentiated stem cells, wherein the cells display the expression of pancreatic cell type markers, including at least one of insulin, Glut-2, Pdx-1, glucokinase, glucagon and somatostatin.

43. The preparation according to claim 42, which is capable of producing at least about 320 ng insulin/mg total protein such as at least about 380 ng insulin/mg total protein or at least about 420 ng insulin/mg total protein.

44. The preparation according to claims 42 or 43, in which preparation the proportion of insulin producing cells is at least 25%, such as e.g. at least 35%, or at least 45%, or at least 50%.

45. The preparation according to claims 42-44, characterized by its organization into islet-like structures comprising an inner core of β-cells surrounded by an outer layer of neuron-type cells, which neuron-type cells display expression of at least one of the following neuronal cell type markers, including neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase and Map 2.

46. The preparation according to claims 42-45, obtained by the method according to claims 36-41.

47. An essentially pure preparation of differentiated stem cells, wherein the cells display the expression of at least one of the following neuronal cell type markers, including neuron-specific β-III tubulin (TUJ1), NeuN, DoubleCortin, tyrosine hydroxylase and Map 2.

48. The preparation according to claim 47, obtained by the method according to claims 36-41.

49. An essentially pure preparation of cells obtainable by the method according to claims 36-41.

50. Use of a preparation according to claims 42-46 for the manufacture of a medicament for the prevention or treatment of pathologies or diseases in the pancreas.

51. Use according to claim 50, in which the disease is diabetes.

52. Use according to claim 50 or 51, in which the disease is type 1 diabetes.

53. Use of a preparation according to claims 47-48 for the manufacture of a medicament for the treatment of pathologies or diseases in the nervous system.

54. Use according to claim 53, in which the disease is selected from the group consisting of multiple schlerosis, spinal chord injury, encephalopathies, Parkinson's disease, Huntingdon's disease, stroke, traumatic brain injuries, hypoxia induced brain injuries, ischemia induced brain injuries, hypoglycemic brain injuries, degenerative disorders of the nervous system, brain tumors and neuropathies in the peripheral nervous system.

55. Kit for performing the method according to claims 36-41, comprising at least two of the following components in separate compartments; mitomycin C, hBS medium, BS cell body medium, ITSFn-medium, N2-medium, B27-media supplement, nicotinamide, and bFGF.

56. Kit according to claim 55, further comprising an essentially pure human blastocyst-derinved stem cell line obtained by the method according to any of the claims 1-20.